cfDNA and Its Applications

What is Cell-Free DNA (cfDNA) or Circulating DNA and Its Applications?

Cell-free DNA (cfDNA) is a term used to describe extracellular DNA fragments in the bloodstream and other bodily fluids. The fragments consist of circulating tumor DNA (ctDNA), cell-free mitochondrial DNA (ccf mtDNA), and cell-free fetal DNA (cffDNA), among others. The hematological system is the primary source of cfDNA molecules in healthy people. However, during certain physiological or pathological situations like pregnancy, cancer, or organ transplantation, affected tissues may release more DNA into the peripheral circulation. The fragments are most commonly found in nucleosomes, which are nuclear aggregates containing histones and DNA.

cfDNA has numerous applications in clinical medicine, especially as a biomarker in cancer diagnostics, prognostics, and therapies. The analysis of cfDNA in different aspects of cancer therapy is becoming increasingly popular, and compared to invasive approaches such as conventional tissue biopsies, examination of cell-free nucleic acids allows for an earlier diagnosis. The amount of cfDNA in plasma depends on various factors, such as the donor's health, blood sample and handling, plasma preparation, DNA isolation method, and other factors. The amount of cell-free DNA in blood plasma is generally low, typically ranging from 0.1 ng to several hundred ng DNA per mL of blood, with an average of 30ng/ml.

To isolate cfDNA, numerous methods and kits are available, and their various procedures and alterations result in a diverse set of cell-free DNA purification processes, which may affect cell-free DNA yield and purity. Therefore, considering the tube type and other pre-analytical variables and downstream analytical methods may help to determine the best DNA purification strategy. Bioclone Cell-Free DNA Purification Kit offers a unique magnetic bead-based purification technology to recover cfDNA from plasma, serum, and urine, which is challenging due to the trace amount of circulating nucleic acid present in cell-free samples. The kit is scalable and allows for multiple samples to be isolated simultaneously when combined with an automation system, and it recovers cfDNA via reversible adsorption of nucleic acids to paramagnetic beads under optimal buffer conditions.

After cfDNA purification, numerous ctDNA assays and procedures are available, which can be divided into targeted assays and broader coverage. Targeted assays are effective in detecting certain known variations in a single gene or a limited group of genes, often at extremely low levels. Targeted assays use one of various polymerase chain reaction (PCR)-based methods, such as real-time or digital PCR, to detect known recurrent somatic variations. Sensitive methods such as quantitative PCR or NGS technologies are then used to detect targets such as miRNA, single nucleotide variations, or chromosomal alterations in the broader coverage analysis.

In conclusion, cfDNA has revolutionized the field of clinical medicine, especially in cancer diagnostics, prognostics, and therapies. Its ease of use, noninvasive nature, and potential tumor specificity through quantitative detection or specific sequencing make it a promising biomarker. With the numerous methods and kits available for cfDNA isolation and analysis, research on cfDNA will continue to progress, leading to more promising clinical applications.

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- One-Step Fluorescent Labeling Cleanup Kit
- One-Step PCR Cleanup Kit
- One-Step NGS Cleanup Kit
- One-Step PCR Inhibitor Removal Kit
- One-Step RNA Removal Kit

General references

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